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(54) Title: USE OF BUCHU EXTRACTS FOR HYPERTENSION

(57) Abstract: A method is described for obtaining a composition for the treatment of inflammatory and/or hypertensive conditions. The plant (*Barosma betulina*, *agosthoma betulina* or *agosthoma crenulata*) is vacuum steam distilled and fractions rich in one or more of diphenol, diosmin, quercetin and rutin are separated and used in dilutions of from 1:400 to 1:3200.

USE OF BUCHU EXTRACTS FOR HYPERTENSION
TECHNICAL FIELD OF THE INVENTION

This invention relates to a composition of matter which is useful in the treatment of hypertension and inflammatory conditions

5 *BACKGROUND ART*

Inflammation and hypertension are two well known conditions and it is not considered necessary to explain, in a patent specification, the medical reactions responsible for the hypertensive and inflammatory conditions.

It is known that oil of buchu (*Barosma betulina* or *agosthoma betulina* or
10 *agosthoma crenulata*) has been used as an anti-inflammatory agent, usually in the form of a tea prepared from the leaves of the plant.

As a result of extensive experimentation, it has surprisingly been found that certain fractions of the distillate of the buchu oil have unexpectedly beneficial properties.

DISCLOSURE OF THE INVENTION

15 According to the invention a composition for the treatment of inflammatory and/or hypertensive conditions includes one or more fractions of the vacuum steam distillation of buchu oil, whether as the raw product obtained directly from the plant or as a residue from a process aimed at the separation of fractions for other purposes, the fraction/s comprising those rich in one or more of diphenol, diosmin, quercetin and rutin.

20 Hesperidin and Vitamin B and E may also be present in substantial quantities.

Such fractions are determined by experimentation and it has surprisingly been shown that such fractions exhibit an action far in excess of other fractions.

As an anti-inflammatory agent, the fraction/s exhibit unexpected action on neutrophil and monocyte functions; whereas as an anti-hypertensive agent the administration leads to unexpectedly good normalisation of blood pressure without any need for additional medication. This may be due to the increased secretion of renin by the kidneys.

- 5 The invention also extends to a method of manufacturing a composition useful in the treatment of inflammatory and/or anti-hypertensive conditions, comprising the steps of selecting one or more fractions of the vacuum stream distillation of buchu oil, the fractions being rich in diphenol, diosmin, quercetin and rutin.

A. ANTI-INFLAMMATORY ACTION

10 EXPERIMENTAL PROCEDURES:

♦ Neutrophil Functions:

e) Adhesion molecules:

15 Upon activation by complement peptides, the neutrophils up-regulate the expression of the adhesion molecules on their surfaces in order to adhere more efficiently to the cell wall of the bacteria and endothelial cells. These adhesion molecules are called the β -Integrins. There are 2 that are present on the cell surfaces of neutrophils and both are expressed constitutionally but up regulated (expressed more) upon activation: the so-called LFA-1 (CD11a/CD18 heterodimer) and the Mac-1 (CD11b/CD18 heterodimer).
20 Both of these surface molecules can be measured and quantified by means of flow cytometry (a specialized technique that measures the amount of binding which takes place between the antibody specific for these molecules and the cell in question).

25 Heparinised blood from healthy volunteers was obtained in vacuutainer tubes. The donating volunteers were not on any anti-inflammatory medication. An aliquot of whole blood was pre-incubated (20 minutes at room temperature) with various dilutions of the Buchu essential oil distillate prior to being stimulated using PMA (phorbol myristate acetate, an activator used *in vitro*). The experimental outline was as follows:

- I. Blood incubated with medium
- II. Blood with stimulant (PMA)
- III. Blood with stimulant (PMA) AND Buchu oil dilution in a increasing doses

5 Measurement of Mac-1 was conducted using antibodies that bind to these structures and analysed by flow cytometry. Briefly, the blood aliquot pre-incubated and activated with the activator was treated with a solution to lyse the red blood cells. The non-lysed cells were washed with an isotonic solution and analysed on a flow cytometry. Fifteen thousand events were accumulated and the % positive events as well as the degree of positivity (mean channel fluorescent: MCF) was measured.

c) Respiratory burst:

10 When the neutrophils arrive at the site of infection, they eat the organisms invading the host and once inside the cytoplasm of the cells, activate a series of enzymes on the inside of the membrane to generate toxic oxygen radicals. This is referred to as the oxygen-dependent killing mechanism. This respiratory burst can be measured by using fluorochromes which change colour when in the presence of the oxygen radicals generated. This change can be quantified by flow cytometry.

15 A commercial kit (BurstTest) was used to measure the ability of neutrophils to mount a respiratory burst. For this, blood was obtained from healthy volunteers as above. The aliquots were pre-incubated with dilutions of the Buchu oils prior to being activated with whole bacteria (included in the commercial kit) and processed further. The experimental outline was as follows:

- 20
- I. Blood with medium
 - II. Blood with activator (*E coli*)
 - III. Blood with activator (as above) AND Buchu oil in increasing doses

Following the pre-incubation and activation, the red blood cells were lysed and the non-lysed cells washed and analysed on a flow cytometry. The % positive cells as well as the MCF measured. Fifteen thousand events were accumulated for the analysis.

25 ♦ **Monocyte Functions:**

a) Expression of adhesion molecules:

30 Similarly to the measurement of adhesion molecules on neutrophils, antibodies binding to the Mac-1 will be used to measure the expression of these molecules when monocytes are activated in the presence/absence of Buchu oil extract (see above). The same reagents are used for this procedure.

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b) Release of pro-inflammatory monokines by monocytes:

When activated, monocytes release IL6 and TNF- α that are responsible for the recruitment of other immune cells and initiate a self-perpetuating chronic inflammatory process. These factors can be quantified by activating monocytes with bacterial products (endotoxin) and measuring the soluble factors by immunological assays (ELISA) using specific antibodies. The aims of these experiments were to measure the release of IL6 in the following way. Whole blood was collected into vacutainer tubes and the mononuclear cells isolated by density centrifugation. The monocyte rich fraction was obtained by adherence to the wells of a multi-well culture plate. The adherent cells were stimulated to release the monokine IL6 by being activated by a pre-determined concentration of bacterial endotoxin (Lipopolysaccharide). The experimental outline was as follows:

- I. Cells with medium
- II. Cells with endotoxin
- III. Cells with endotoxin with Buchu oil in increasing doses

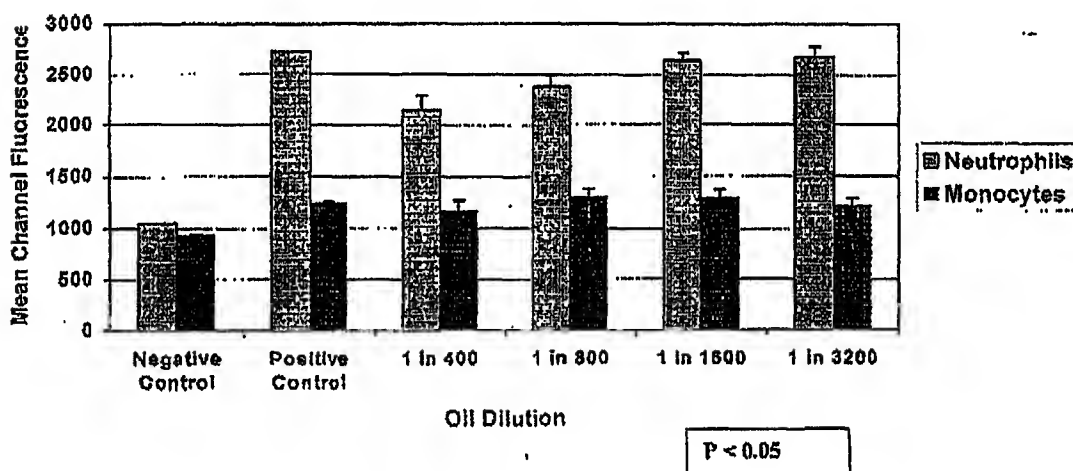
The cells were incubated for 17 hours at 37°C and 5% CO₂. The following day, the supernatant was collected and assayed for their contents in IL6 by an in-house ELISA method using external standards as calibrators. The absolute concentrations of IL6 in the respective supernatants were calculated from a standard curve derived from the standards. The results were expressed as Units of IL6 (pg/ml) released by the respective culture wells.

RESULTS:**c) Expression of Adhesion molecules (MAC-1) on Neutrophils and Monocytes:**

As shown in Figure 1, the neutrophils pre-incubated with the various dilutions of Buchu oil distillate showed a statistically significant decrease in their ability to express the

MAC-1 adhesion molecules as measured by flow cytometry. Indeed, when the cells are activated by PMA, they increase their expression of MAC-1 (compare negative control versus positive control). When the cells are activated in the presence of increasing dilutions of the Buchu oil, the expression is significantly inhibited ($p < 0.05$) by dilutions of 1:400; 1:800 and 1:1600. Thereafter, the inhibitory effects are diluted out. This effect was not paralleled by the monocytes under identical conditions of culture.

Figure 1: Expression of MAC-1 (CD11b/CD18) by Neutrophils and monocytes post activation in the presence/absence of Buchu oil distillate at different dilutions:

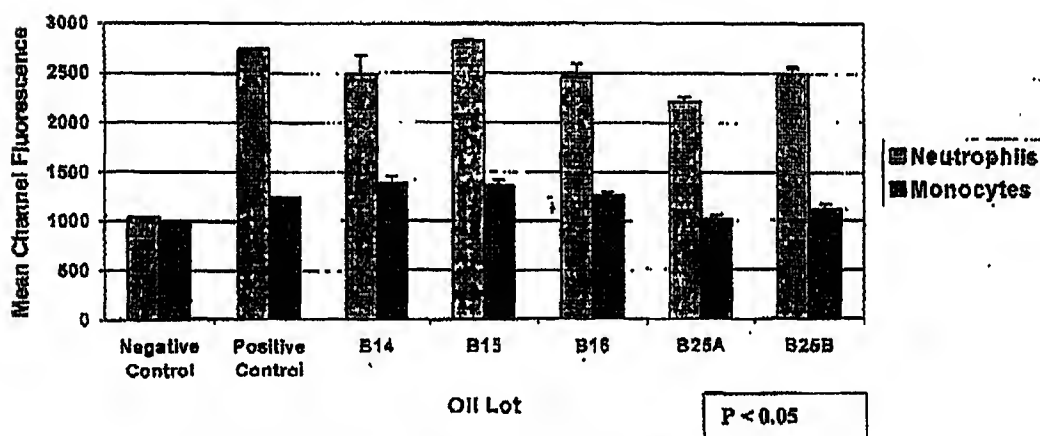


e) Effects of different Buchu oil distillates on the expression MAC-1 in vitro:

Although most of the oil distillates tested yielded inhibition of the MAC-1 expression by neutrophils, it was evident when each oil was tested separately that some of the oils had higher activity when analysed separately. As indicated in Figure 2, some oils were more potent inhibitors of the expression of MAC-1 upon stimulation by PMA. The same experimental procedure was followed as above.

It can be seen from Figure 2 that certain preparations of the distillate are more effective in the inhibition of MAC-1 expression: B16 and B25A show higher inhibition than the other 3 batches of oils tested.

Figure 2: The effects of different oil distillates at the same dilution (1:400) on the expression of MAC-1 (CD11b/CD18) on neutrophils and monocytes post-activation by PMA:

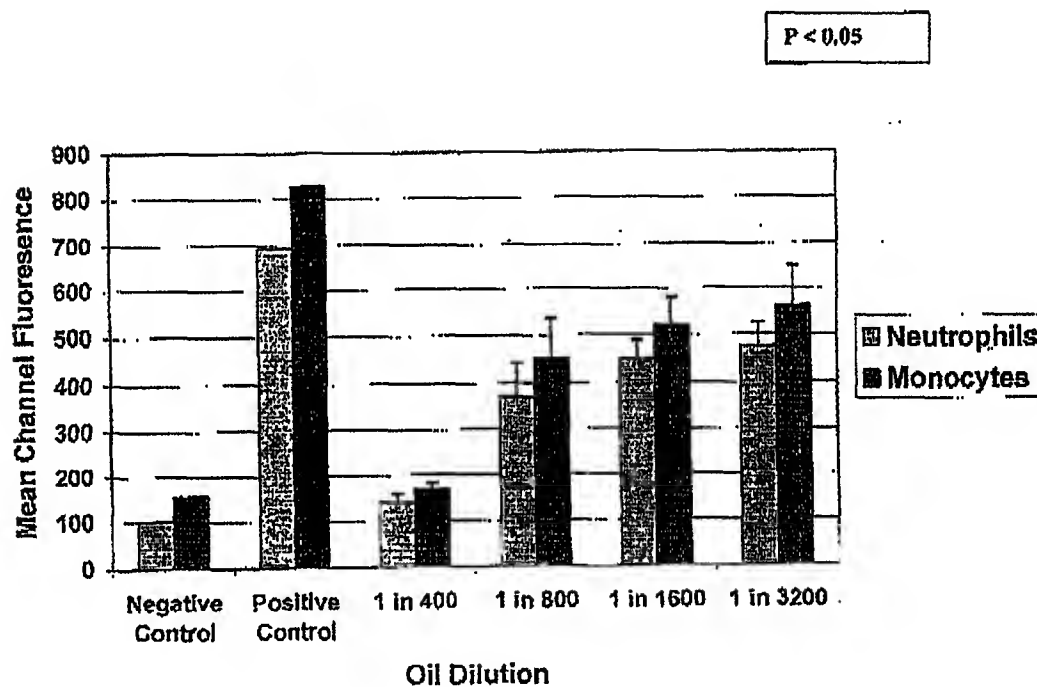


e) The effects of Buchu oil distillates on the Respiratory burst of neutrophils:

During the initial phase of neutrophil functions, the internalised organism or particle is destroyed by an oxygen-dependent process which ultimately leads to the generation of oxygen radicals (O_2^-). These radicals are protective when this is generated in the cytoplasm of the cell in that it kills the bacterium. However, when these are released to the external medium, it can damage healthy tissues in the vicinity of the phagocytic cell.

The ability of Buchu oils to influence the generation of oxygen radicals was measured by flow cytometry. As shown in Figure 3, it is evident that at the different dilutions of the oils, the oxidative burst (respiratory burst) of neutrophils was significantly inhibited by the oil distillate dilutions (from 1:400 to 1:3200 dilution).

Figure 3: Inhibition of Respiratory Burst of neutrophils by different Buchu oil dilutions.



e) Effects of various Buchu oil distillates on the Respiratory Burst of Neutrophils:

It was evident during the measurement of this cellular function that certain batches were more potent in their inhibitory ability. This is shown in Figure 4 where various oil distillates were compared at the same dilution (1:400). It is obvious that batch B16, B25A and B25B were the most effective in this aspect although all had inhibitory activities ($p < 0.05$)

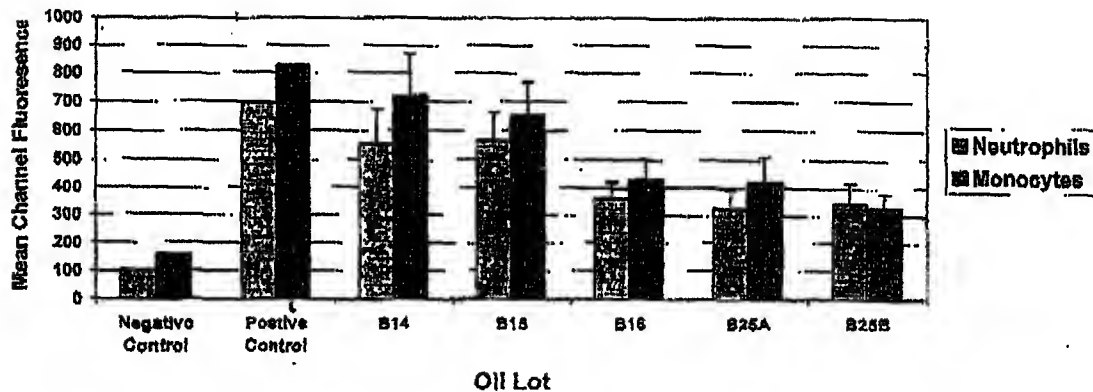


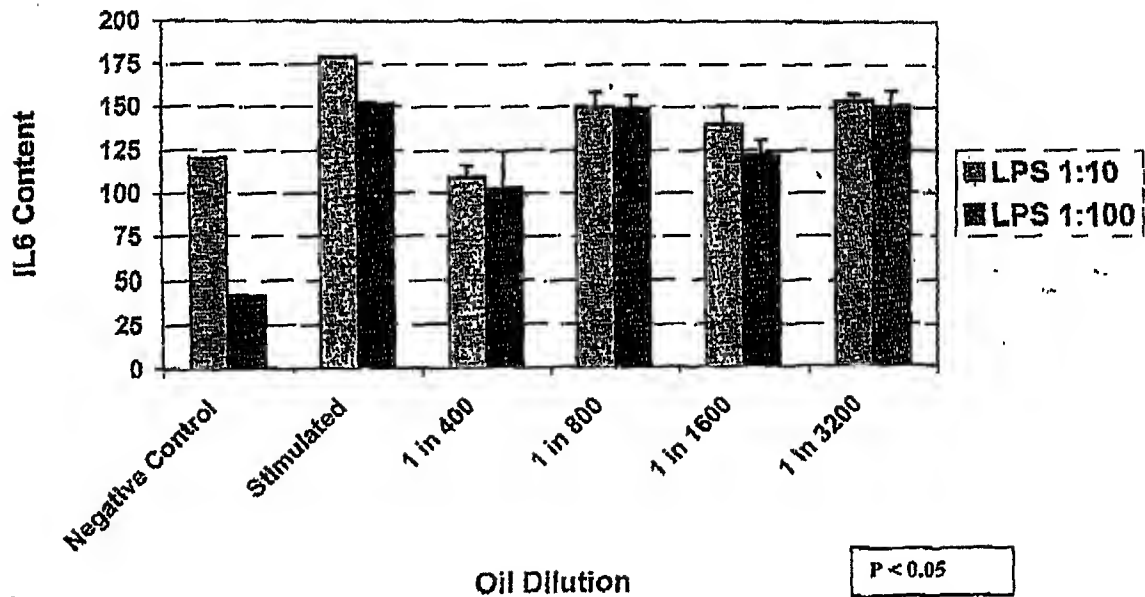
Figure 4: Effects of different Buchu oil distillates on the Respiratory Burst of Neutrophils: all oils were tested at the same dilution (1:400).

e) The effects of Buchu oil distillate on the release of IL6 by activated monocytes:

5 During the chronic phases of inflammation, the cellular infiltrate made up primarily of monocytes and other mononuclear cells release IL6, a factor with varied biological activities including fever, pain, calcium mobilization from bone, etc. This factor perpetuates the on-going inflammation and thus is pivotal in the tissue damage seen in these chronic conditions.

10 Blood monocytes were pre-incubated with various dilutions of Buchu oil prior to being activated by endotoxin in order to induce the synthesis and release of IL6. As shown in Figure 5, it is evident that various dilutions of the Buchu oils were able to significantly inhibit the release of IL6 from monocytes.

Figure 5: Inhibition of IL6 release from monocytes in the presence of Buchu oil distillate:



At both stimulus concentration (LPS 1:100 and LPS 1:10), the 1:400 dilution was the most active in its inhibition. Once the oil was diluted out, this activity was lost.

DISCUSSION:

It has been shown by the present report that various Buchu oil distillates are able to inhibit various processes involved during inflammation. The acute phase of the inflammatory process as measured by the expression of adhesion molecules such as MAC-1 (CD11b/CD18) on both monocytes and neutrophils as well as the respiratory burst of neutrophils were significantly inhibited by low dilutions of the oils tested. This inhibition was dose dependent since titrating out of the oil lead to this effect being lost. Some oils were more effective in this process.

One can mimic the chronic phase of inflammation by inducing and measuring the release of IL6 from activated monocytes. This was measured in the presence of Buchu oil and shown to be significantly inhibited at low dilutions of the oils.

B. ANTI-HYPERTENSIVE ACTION

Experimental Cases:

Case 1:

A 70 year old woman (RH) who for 2 years suffered from high blood pressure. She started drinking 250 ml of Buchu water (an oil extract of the Buchu plant mixed into spring water) each day and within a week, her blood pressure dropped from 150/90 to a more acceptable 140/80 (normal considering the age of this patient).

Case 2:

Mrs GK: suffered from high blood pressure for several years (4 years) necessitating medication which could not control this chronic condition. Several changes of her medication still did not manage to control her blood pressure which stood at 220/120. Four years later, she was introduced to the Buchu water. Within 2 months of using this product regularly, her hypertension has been controlled at 145/80 without any other medication for this serious condition.

Case 3: Mrs DG

This patient was diagnosed with a blood pressure of 160/95 and at other times 170/100. She was prescribed various medications that caused side effects and she was advised to stop the medication. She discovered the Buchu water product and since using the product, her condition improved: her blood pressure now stands at 130/75. She has since never shown signs of her previously chronic high blood pressure.

CLAIMS

1. A method of preparing a composition for the treatment of inflammatory and/or hypertensive conditions characterised in that buchu oil or a product containing buchu oil is vacuum steam distilled, those fractions which are rich in one or more of diphenol, diosmin, quercetin and rutin being separated and incorporated into a pharmaceutically acceptable form.
5
2. The method according to claim 1 characterised in that the fractions separated also contain one or more of hesperidin, Vitamin B and vitamin E.
3. The method according to either claim 1 or claim 2 characterised in that the fractions are diluted by a factor of between 1:400 to 1:3200.
- 10 4. A pharmaceutical composition for the treatment of inflammatory and/or hypertensive conditions characterised by including one or more fractions of the vacuum steam distillation of buchu oil, whether as the raw product obtained directly from the plant or as a residue from a process aimed at the separation of fractions for other purposes, the fraction/s comprising those rich in one or more of diphenol, diosmin, quercetin and rutin.
- 15 5. The composition according to claim 4 characterised in that the fraction/s also contain one or more of hesperidin, Vitamin B, and vitamin E.
6. A method of treating a subject suffering from inflammatory and/or hypertensive conditions characterised in the administration of a pharmaceutically sufficient amount of a composition according to claim 4 or claim 5.